EFFECTIVENESS OF DEET AND PERMETHRIN, ALONE AND IN A SOAP FORMULATION AS SKIN AND CLOTHING PROTECTANTE AGAINST MOSQUITOES IN AUSTRALIA.¹

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This note reports studies conducted in Australia to study the effectiveness of a new mosquito repellent formulation containing 20% N,N-diethyl-m-toluamide (deet) and 0.5% permethrin in a bar of soap. The active ingredients are not new. Deet has gained wide acceptance and is used in many countries throughout the world (Rutledge et al. 1978a). Its effects against many arthropod species have been studied, and these have shown it is effective against a number of insects genera (Rutledge et al. 1978b, Schreck 1977). More recently, the effectiveness of permethrin as a clothing impregnation or pressurized spray for personal protection against mosquitoes, ticks and biting flies has been demonstrated (Lindsay and McAndless 1978; Shreck et al.1982, 1984,1986). The soap formulation warranted study as it is cheap to produce; a 70g block costing about \$US 0.25, and encouraging results have been reported against mosquitoes in Malaysia (Yap 1986).

Two field trials were conducted. An initial trial compared the effects of the soap containing deet and permethrin with that of 95% deet alone on day biting mosquitoes in a temperate salt marsh. A second trial compared the soap and deet formulation alone, and in combination with two clothing impregnations for personal protection against day-biting mosquitoes in a tropical rain forest. During this trial the effects of deet alone and the soap formulation on crepuscular mosquitoes was also compared.

The first trial was conducted at Powells Creek Reserve, Homebush Bay, New South Wales in January 1986. The area is a salt marsh wetland with Salicornia sp. and mangrove being the main plant types present. Samples taken before and during this trial revealed the mosquito population was almost entirely (>99%) Aedes vigilax (Skuse). Six male volunteers were used during this trial. For the duration of the trial the volunteers were Australian Army jungle greens, made of 100% cotton fabric, with trouser legs bloused and shirt sleeves rolled to the elbow. The Australian Army personal insect repellent formulation of 95% deet and 5% ethanol, and the soap formulation were compared during this trial. Each of the subjects using deet applied 3ml to the hands, arms, neck and face. The soap formulation was applied to exposed skin by welting the skin with a few drops of water and lathering with soap to ensure a uniform coverage. The later was allowed to dry leaving a soap residue on skin, deet on skin and no treatment (control) with this two subjects per treatment. Each pair of subjects evaluated a different treatment on each of the three test days. The repellents were applied at 0845 hours. The volunteers walked slowly through the test site, with short rests in circuits of up to 600 meters. The mosquitoes coming to bite on skin or landing on the untreated clothes were collected using aspirators, and placed into separate cups. Each volunteer collected mosquitoes from his partner. The time taken for each circuit was 30 to 45 min, depending on mosquito density, and circuits commenced at 0, 1, 2, and 4 hr after repellent application. Mosquito biting rates on skin and landing rates on clothing for each hour of collecting were calculated. The second trial was conducted in a rain forest at the foot of Brown Range, Cowley Beach, northern Queensland in April 1986. During this study 90% of the mosquitoes collected during the day were *Aedes (Verralina)* sp., the blacklegged *Aedes*, which occur in coastal jungles in Queensland, and are voracious biters (Marks 1982). Smaller numbers o *Aedes kochi* (Dönitz) (3%) and *Aedes notoscriptus* (Skuse) (3%) adults were also collected.

Ten male volunteers wore Australian Army jungle greens in the same manner as Trial 1. The repellent formulations of 95% deet and the soap formulation were compared, and were applied in the same way as in Trial 1. During this trial two clothing impregnations were also compared. Permethrin from Peregrin®, a 10% EC supplied by Wellcome Australia, was prepared as a 1:49 emulsion in water. Four sets of jungle greens were placed into the emulsion for 2 minutes, completely saturated and removed to dry. Uniforms were stored on hangers until use. Other sets of clothes were treated with the soap formulation. Each item of clothing was moistened and then thoroughly washed with the soap formulation; the equivalent of 210g were used to treat four sets of jungle greens. The clothes were allowed to dry, leaving a soap residue on the fabric. These treatments were performed to test the effectiveness of the impregnations in an operational test, and it was not possible to confirm the amount or uniformity of active ingredient added to each item of clothing.

Five treatments evaluated at Cowley Beach during day tests were soap on skin/untreated clothes, deet on skin/untreated clothes, soap on skin/soap-treated clothes, deet on skin/permethrin-treated clothes and no treatment (control). For this tests the repellents were applied at 0745 hours. Collections were commenced at 0, 1, 3 and 6 hr postrepellent application. The test methods used in Trial 1 were used during this trial.

In addition to daytime trials at Cowley Beach a study of the effect of the deet formulations on crepuscular mosquitoes was conducted. The active mosquito species collected during these dusk studies were Ae. kochi (34.1%), Culex annulirostris Skuse (20.2%) and Anopheles farauti s.l. (6.2%), while the day-biting Ae. (Verralina) sp. (23.8%)were also collected, but were less active after dusk. During these studies groups of three volunteers evaluated the three treatments of soap on skin, 95% deet on skin and no treatment (control). All volunteers were dressed in the same way as in the day trials, and the uniforms were untreated. The test repellents were applied at 1745 hr and collections were made for 30-min periods between 1800 and 1930 hr on each of four evenings. The volunteers sat in groups of three in locations in the forest, with groups changing location every 20 minutes. Mosquitoes coming to bite at the skin were collected from each man by his partners using aspirators. The total number of mosquitoes coming to bite the exposed skin for each group was recorded for each treatment.

The mosquito biting density did not vary greatly for the duration of each of the trials conducted. In all trials the data for all mosquito species were combined. In each trial volunteers who received repellent treatments received who fewer bites than untreated volunteers (Tables 1, 2 and 3). Deet was effective in repelling day-biting mosquitoes, in both localities, for up to 5 hours postrepellent application. Percentage repellency was calculated for each treatment using the formula of Mehr et al. (1985) who converted the

number of bites on treated subjects to percentages of the total for the control, and subtracted this from 100 . At Powells Creek, deet had 99.9% repellency after 5 hours and at Cowley Beach showed 97.9% after 7 hours when worn with untreated clothing. The deet-permethrin soap formulation was also effective in reducing mosquito biting, but was less effect than deet, with a 90% repellency at Powells Creek, and 82.9% repellency at Cowel Beach. This was possibly due to an inability to obtain a complete coverage of the skin with the soap, which may be an inherent limitation of this formulation. Also the soap is susceptible to abrasion thereby reducing its time of effective action.

Table 1. Mean bites/hour on treated skin, and mean landing/hour on untreated clothes of Aedes vigilax at Powells Creek, January 1986*

	Site of	Hours posttreatment				
Treatment	collectio	0	1	2	4	
	n					
Deet-permethrin	Skin	0	1.7 ± 1.7	3.3 ± 2.9	4.0 ± 0.6	
soap						
	Clothes	40.3 ± 24.4	43.0 ± 22.0	49.7 ± 26.5	51.0 ± 6.2	
95% deet	Skin	0	0	0	0.3 ± 0.3	
	Clothes	17.0 ± 6.1	18.3 ± 8.9	32.3 ± 7.9	54.0 ± 5.5	
Control	Skin	20.3 ± 5.2	21.0 ± 10.8	27.7 ± 6.2	21.7 ± 2.9	
	Clothes	65.0 ± 22.0	83.3 ± 43.6	83.7 ± 14.9	65.0 ± 14.4	

^{*} Means of 3 replications \pm SE.

Table 2. Mean bites/hr on skin, and mean landings/hr on clothes of day-biting mosquitoes for five treatments

At Cowley Beach, April 1986*

	Site of	Hours posttreatment				
Treatment	collecti	0	1	2	3	6
	on					
Deet-permethrin	Skin	1.3 ± 1.3	6.5 ± 2.5	6.0 ± 1.2	4.9 ± 0.9	$11.6 \pm$
soap/untreated						3.8
clothes						
	Clothe	$87.1 \pm$	$129.3 \pm$	117.1 ± 30.4	$86.7 \pm$	$75.1 \pm$
	S	14.1	37.7		28.0	17.5
95% deet/untreated	Skin	0.4 ± 0.4	0	0	0	3.3 ± 3.3
clothes						
	Clothe	61.3 ±	43.6 ± 6.3	36.7 ± 15.8	46.4 ±	84.9 ±
	S	14.6			11.5	27.0
Deet-permethrin	Skin	3.1 ± 0.9	7.1 ± 5.2	2.0 ± 2.0	4.9 ± 3.6	15.8 ±

soap/soap treated clothes						4.9
	Clothe	77.3 ±	32.9 ±	44.6 ± 32.6	33.6 ±	84.2 ±
	S	51.4	24.3		24.6	41.4
95%	Skin	0	0	0	0.4 ± 0.4	4.7 ± 2.9
deet/permethrin						
treated clothes						
	Clothe	$27.5 \pm$	$14.2 \pm$	5.3 ± 5.3	7.1 ± 1.5	$29.2 \pm$
	S	26.3	12.9			11.7
Control	Skin	41.9 ± 4.6	34.9 ± 3.5	16.2 ± 7.1	$47.8 \pm$	$37.8 \pm$
					15.4	16.3
	Clothe	69.3 ±	58.9 ±	41.8 ± 14.2	71.3 ±	67.8 ±
	S	11.3	14.1		23.1	23.5

^{*}Mean of 3 replications \pm SE.

Table 3. Mean bites of crepuscular mosquitoes on repellent-treated skin at Cowley Beach, April 1986.*

	Period of collection (hr)				
Treatment	1800-1830	1830-1900	1900-1930		
Deet-permethrin soap	1.3 ± 0.5	1.8 ± 0.6	0.8 ± 0.5		
95% deet	0	0	0		
Control	54.8 ± 7.1	54.8 19.4	43 ± 19.6		

^{*} Means of 4 replications \pm SE.

Both repellents were effective against crepuscular mosquitoes at Cowley Beach (Table 3). The small number of mosquitoes coming to bite volunteers treated with soap were collected from ears and finger tips, where a complete coverage of repellent could not be achieved. Mosquitoes did not bite individuals treated with 95% deet during these dusk studies.

In both day trials mosquitoes were collected landing on clothing. Both Ae. vigilax and Ae. (Verralina) sp. landed on untreated clothing and began probing, but only succeeded in obtaining a blood meal if the clothing was taut on the skin. Effects of clothing impregnations were observed during day tests at Cowley Beach (Table 2). The number of mosquitoes collected on soap-washed clothing did not differ markedly from those collected on untreated clothing. This was not unexpected as little active ingredient was applied to each item of clothing despite thorough washing. The use of permethrin as a clothing impregnation was shown to be effective, with fewer mosquitoes landing on treated clothing (Table 2). Mosquitoes were observed attempting to alight on permethrin-treated clothing, but they quickly left the treated surface.

The results of the current trials confirm the repellency of the Australian Army deet formulation. The soap formulation also utilizes deet, and, although it is cheaper to produce than other deet formulation may be utilized in other situation against pest mosquitoes, and has been reported to be effective against outdoor mosquitoes in Penang, Malaysia (Yap 1986). The results of the Cowley Beach trial support the findings of other workers who showed that permethrin is an effective clothing impregnation for protection against mosquitoes (Lindsay and McAndless 1978, Schreck et al. 1984).

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